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(54) Title: IL-1 ETA DNA AND POLYPEPTIDES

(57) **Abstract:** The invention is directed to novel, purified and isolated IL-1 eta polypeptides and fragments thereof, the polynucleotides encoding such polypeptides, processes for production of recombinant forms of such polypeptides, antibodies generated against these polypeptides, fragmented peptides derived from these polypeptides, and uses thereof.

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TITLE

IL-1 ETA DNA AND POLYPEPTIDES

BACKGROUND OF THE INVENTION

Field of the Invention

The invention is directed to novel, purified and isolated IL-1 eta polypeptides and fragments thereof, the polynucleotides encoding such polypeptides, processes for production of recombinant forms of such polypeptides, antibodies generated against these polypeptides, fragmented peptides derived from these polypeptides, and uses thereof.

Description of Related Art

Interleukin-1 (IL-1) is a member of a large group of cytokines whose primary function is to mediate immune and inflammatory responses. There are seven known IL-1 family members which include IL-1 alpha (IL-1α), IL-1 beta (IL-1β), IL-1 receptor antagonist (IL-1ra), IL-1 delta (IL-1δ), IL-1 epsilon (IL-1ε), IL-1 zeta (IL-1ξ) and IL-18 (previously known as IGIF and sometimes IL-1 gamma). IL-1 that is secreted by macrophages is actually a mixture of mostly IL-1β and some IL-1α (Abbas et al., 1994). IL-1α and IL-1β, which are first produced as 33 kD precursors that lack a signal sequence, are further processed by proteolytic cleavage to produce secreted active forms, each about 17 kD. Additionally, the 33 kD precursor of IL-1α is also active. Both forms of IL-1 are the products of two different genes located on chromosome 2. Although the two forms are less than 30 percent homologous to each other, they both bind to the same receptors and have similar activities.

IL-1ra, a biologically inactive form of IL-1, is structurally homologous to IL-1 and binds to the same receptors. Additionally, IL-1ra is produced with a signal sequence that allows for efficient secretion into the extracellular region where it competitively competes with IL-1 (Abbas et al., 1994).

The IL-1 family of ligands binds to a family of two IL-1 receptors, which are members of the Ig superfamily. IL-1 receptors include the 80 kDa type I receptor (IL-1RI) and a 68 kDa type II receptor (IL-1RII). IL-1 ligands can also bind to a soluble proteolytic fragment of IL-1RII (sIL-1RII) (Colotta et al., 1993).

The major source of IL-1 is the activated macrophage or mononuclear phagocyte. Other cells that produce IL-1 include epithelial and endothelial cells (Abbas et al., 1994). IL-1 secretion from macrophages occurs after the macrophage encounters and ingests gram-negative bacteria. Such bacteria contain lipopolysaccharide (LPS) molecules, also known as endotoxin, in the bacterial cell wall. LPS molecules are the active components that stimulate macrophages to produce tumor necrosis factor (TNF) and IL-1. In this case, IL-1 is produced in response to LPS and TNF production. At low concentrations, LPS stimulates macrophages

and activates B-cells and other host responses needed to eliminate the bacterial infection; however, at high concentrations, LPS can cause severe tissue damage, shock, and even death.

The biological functions of IL-1 include activating vascular endothelial cells and lymphocytes, local tissue destruction, and fever (Janeway et al., 1996). At low levels, IL-1 stimulates macrophages and vascular endothelial cells to produce IL-6, upregulates molecules on the surface of vascular endothelial cells to increase leukocyte adhesion, and indirectly activates inflammatory leukocytes by stimulating mononuclear phagocytes and other cells to produce certain chemokines that activate inflammatory leukocytes. Additionally, IL-1 is involved in other inflammatory responses such as induction of prostaglandins, nitric oxide synthetase, and metalloproteinases. These IL-1 functions are crucial during low level microbial infections. However, if the microbial infection escalates, IL-1 acts systemically by inducing fever, stimulating mononuclear phagocytes to produce IL-1 and IL-6, increasing the production of serum proteins from hepatocytes, and activating the coagulation system. Additionally, IL-1 does not cause hemorrhagic necrosis of tumors, suppress bone marrow stem cell division, and IL-1 is lethal to humans at high concentrations.

EP 0 879 889 A2 discusses the multiple uses of an example member of the IL-1 family. In this publication, it is disclosed that IL-1 delta polypeptides may be used for the treatment of various diseases, including chronic and acute inflammation, arthritis, septicemia, autoimmune disease, transplant rejection, graft versus host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, bone diseases, cancer, atherosclerosis and Alzheimer's disease. Other diseases for which IL-1 family members may be used for treatment of are inflammatory bowel disease, multiple myeloma, multiple sclerosis, asthma, allergy, osteoporosis, pancreatitis, acute myelogenous leukemia, chronic myelogenous leukemia, heart disease including myocardial infarction and congestive heart failure, Lyme disease, periodontal disease, sepsis, heat stroke, glomerulonephritis, osteoarthritis, granuloma formation, pre-term labor and uveitis.

Given the important function of IL-1, there is a need in the art for additional members of the IL-1 ligand and IL-1 receptor families. In addition, in view of the continuing interest in protein research and the immune system, the discovery, identification, and roles of new proteins (such as the human IL-1 eta of the invention) and their inhibitors, are at the forefront of modern molecular biology and biochemistry. Despite the growing body of knowledge, there is still a need in the art for the identity and function of proteins involved in cellular and immune responses.

SUMMARY OF THE INVENTION

The invention aids in fulfilling these various needs in the art by providing the isolated polynucleotides and the polypeptides encoded by these polynucleotides for the novel IL-1 family ligand termed "IL-1 eta." Thus, in one aspect, the invention is directed to isolated novel polynucleotide molecules of IL-1 eta comprising the DNA sequence of SEQ ID NO:1 and to the isolated polynucleotide molecules encoding the amino acid sequence of SEQ ID NO:2, as well as polynucleotide molecules complementary to these sequences.

Both single-stranded and double-stranded RNA and DNA polynucleotide molecules are encompassed by the invention, as well as polynucleotide molecules that hybridize to a denatured, double-stranded DNA comprising all or a portion of SEQ ID NO:1 and/or a DNA that encodes the amino acid sequences set forth in SEQ ID NO:2. Also encompassed are isolated polynucleotide molecules that are derived by *in vitro* mutagenesis of polynucleotide molecules comprising the sequence of SEQ ID NO:1, that are degenerate from polynucleotide molecules comprising the sequence of SEQ ID NO:1, and that are allelic variants of DNA of the invention. The invention also encompasses recombinant vectors that direct the expression of these polynucleotide molecules and host cells transformed or transfected with these vectors.

In addition, the invention encompasses methods of using the polynucleotides noted above to identify polynucleotides encoding proteins having activities associated with IL-1 family ligands and receptors. Thus, the IL-1 eta polynucleotides can be used to identify the IL-1 eta receptor by coding for the IL-1 eta protein.

In addition, these polynucleotides can be used to identify the human chromosomes with which the polynucleotides are associated. Thus, the IL-1 eta polynucleotides may be used to identify human chromosome 2. Accordingly, these polynucleotides may also be used to map genes on human chromosome 2; to identify genes associated with certain diseases, syndromes, or other human conditions associated with human chromosome 2; and to study cell signal transduction and the immune system.

The invention also encompasses the use of sense or antisense oligonucleotides from the polynucleotides of SEQ ID NO:1 to inhibit the expression of the respective polynucleotide encoded by the genes of the invention.

The invention also encompasses isolated polypeptides and fragments of IL-1 eta as encoded by these polynucleotide molecules, including soluble polypeptide portions of SEQ ID NO:2. The invention further encompasses methods for the production of these polypeptides, including culturing a host cell under conditions promoting expression and recovering the polypeptide from the culture medium. Especially, the expression of these polypeptides in bacteria, yeast, plant, insect, and animal cells is encompassed by the invention.

In general, the polypeptides of the invention can be used to study cellular processes such as immune regulation, cell proliferation, cell death, cell migration, cell-to-cell interaction, and inflammatory responses. In addition, these polypeptides can be used to identify proteins associated with IL-1 eta ligands.

In addition, the invention includes assays utilizing these polypeptides to screen for potential inhibitors of activity associated with polypeptide counter-structure molecules, and methods of using these polypeptides as therapeutic agents for the treatment of diseases mediated by polypeptide counter-structure molecules. Further, methods of using these polypeptides in the design of inhibitors (e.g., engineered receptors that act as inhibitors) thereof are also an aspect of the invention.

Further encompassed by this invention is the use of the IL-1 eta polynucleotide sequences, predicted amino acid sequences of the polypeptide or fragments thereof, or a combination of the predicted amino acid sequences of the polypeptide and fragments thereof for use in searching an electronic database to aid in the identification of sample polynucleotides and/or proteins.

Isolated polyclonal or monoclonal antibodies that bind to these polypeptides are also encompassed by the invention, in addition the use of these antibodies to aid in purifying the polypeptides of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The polynucleotide molecules encompassed in the invention include the following nucleotide sequence:

Name: IL-1 eta

1 GGCACGAGGT TCCTCCCCAC TCTGTCTTTC TCACCTCTCC TTCACTTTTC
51 CTAGCCTCCT CACCACCATC TGATCTATCT TGTTCTCTTC ACAAAAGGCT
101 CTGAAGACAT CATGAACCCA CAACGGGAGG CAGCACCCAA ATCCTATGCT
151 ATTCGTGATT CTCGACAGAT GGTGTGGGTC CTGAGTGGAA ATTCTTTAAT
201 AGCAGCTCCT CTTAGCCGCA GCATTAAGCC TGTCACTCTT CATTTAATAG
251 CCTGTAGAGA CACAGAATTC AGTGACAAGG AAAAGGGTAA TATGGTTTAC
301 CTGGGAATCA AGGGAAAAGA TCTCTGTCTC TTCTGTGCAG AAATTCAGGG
351 CAAGCCTACT TTGCAGCTTA AGGAAAAAAA TATCATGGAC CTGTATGTGG
401 AGAAGAAAGC ACAGAAGCCC TTTCTCTTTT TCCACAATAA AGAAGGCTCC
451 ACTTCTGTCT TTCAGTCAGT CTCTTACCCT GGCTGGTTCA TAGCCACCTC
501 CACCACATCA GGACAGCCCA TCTTTCTCAC CAAGGAGAGA GGCATAACTA
551 ATAACACTAA CTTCTACTTA GATTCTGTGG AATAA **(SEQ ID NO:1)**

The amino acid sequence of the polypeptides encoded by the nucleotide sequence of the invention include:

Name: IL-1 eta (polypeptide)

1 MNPQREAAPK SYAIRDSRQM VWVLSGNSLI AAPLSRSIKP VTLHLIACRD

51 TEFSDKEKGN MVYLGIKGKD LCLFCAEIQG KPTLQLKEKN IMDLYVEKKA

101 QKPFLFFHNK EGSTSVFQSV SYPGWFIATS TTSGQPIFLT KERGITNNTN

151 FYLDSVE(**SEQ ID NO:2**)

The discovery of the IL-1 eta polynucleotides of the invention enables the construction of expression vectors comprising polynucleotide sequences encoding the respective polypeptides and host cells transfected or transformed with the expression vectors. The invention also enables the isolation and purification of biologically active IL-1 eta polypeptides and fragments thereof. In yet another embodiment, the polynucleotides or oligonucleotides thereof can be used as probes to identify polynucleotide encoding proteins having associated activities. Thus, IL-1 eta can be used to identify activities associated with IL-1 family ligands. In addition, the polynucleotides or oligonucleotides thereof of IL-1 eta may be used to identify human chromosomes 2. Similarly, these polynucleotides or oligonucleotides thereof may be used to map genes on human chromosome 2, and to identify genes associated with certain diseases, syndromes or other human conditions associated with human chromosome 2. Thus, the polynucleotides or oligonucleotides thereof of IL-1 eta may be used to identify glaucoma, ectodermal dysplasia, insulin-dependent diabetes mellitus, wrinkly skin syndrome, T-cell leukemia/lymphoma, and tibial muscular dystrophy. Finally, single-stranded sense or antisense oligonucleotides from these polynucleotides can be used to inhibit expression of polynucleotides encoded by the IL-1 eta.

The IL-1 eta and soluble fragments thereof can be used to activate and/or inhibit the activation of vascular endothelial cells and lymphocytes, induce and/or inhibit the induction of local tissue destruction and fever (Janeway et al., 1996), inhibit and/or stimulate macrophages and vascular endothelial cells to produce IL-6, induce and/or inhibit the induction of prostaglandins, nitric oxide synthetase, and metalloproteinases, and upregulate and/or inhibit the upregulation of molecules on the surface of vascular endothelial cells. In addition these polypeptides and fragmented peptides can also be used to induce and/or inhibit the induction of inflammatory mediators such as transcription factors NF-κB and AP-1, MAP kinases JNK and p38, COX-2, iNOS, and all of the activities stimulated by these molecules.

In addition, these polypeptides and fragmented peptides can be used as molecular weight markers and as controls for peptide fragmentation, and the invention includes the kits comprising these reagents. Finally, these polypeptides and fragments thereof can be used to generate antibodies, and the invention includes the use of such antibodies to purify IL-1 eta polypeptides.

POLYNUCLEOTIDE MOLECULES

In a particular embodiment, the invention relates to certain isolated nucleotide sequences that are free from contaminating endogenous material. A "nucleotide sequence" refers to a polynucleotide molecule in the form of a separate fragment or as a component of a larger polynucleotide construct. The polynucleotide molecule has been derived from DNA or RNA isolated at least once in substantially pure form and in a quantity or concentration enabling identification, manipulation, and recovery of its component nucleotide sequences by standard biochemical methods (such as those outlined in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989)). Such sequences are preferably provided and/or constructed in the form of an open reading frame uninterrupted by internal non-translated sequences, or introns, that are typically present in eukaryotic genes. Sequences of non-translated DNA can be present 5' or 3' from an open reading frame, where the same do not interfere with manipulation or expression of the coding region. Preferably, DNA of the present invention encompasses nucleotides 112 through 585 of SEQ ID NO:1.

Polynucleotides of the invention include DNA in both single-stranded and double-stranded form, as well as the RNA complement thereof. DNA includes, for example, cDNA, genomic DNA, chemically synthesized DNA, DNA amplified by PCR, and combinations thereof. Genomic DNA may be isolated by conventional techniques, *e.g.*, using the cDNA of SEQ ID NO:1, or a suitable fragment thereof, as a probe.

The DNA molecules of the invention include full length genes as well as polynucleotides and fragments thereof. The full length gene may include the N-terminal signal peptide. Other embodiments include DNA encoding a soluble form, *e.g.*, encoding the extracellular domain of the protein, either with or without the signal peptide.

The polynucleotides of the invention are preferentially derived from human sources, but the invention includes those derived from non-human species, as well.

Encompassed by the present invention are cDNA clones having the nucleotide sequence of SEQ ID NO:1 isolated as described in Example 1. The polypeptide encoded by the nucleotides 112-585 of SEQ ID NO:1 is shown in SEQ ID NO:2. This sequence identifies the IL-1 eta of SEQ ID NO:2 as a member of the IL-1 family.

Due to the known degeneracy of the genetic code, wherein more than one codon can encode the same amino acid, a DNA can vary from that shown in SEQ ID NO:1, and still encode a polypeptide having the amino acid sequence of SEQ ID NO:2. Such variant DNA can result from silent mutations (*e.g.*, occurring during PCR amplification), or can be the product of deliberate mutagenesis of a native sequence.

The invention thus provides isolated DNA selected from: (a) DNA comprising the nucleotide sequences of SEQ ID NO:1; (b) DNA comprising nucleotides 112-585 of SEQ ID

NO:1; (c) DNA encoding the polypeptides of SEQ ID NO:2; (d) DNA capable of hybridization to a DNA of (a)-(c) under conditions of moderate stringency and which encodes polypeptides of the invention; (e) DNA that is the complement of capable of hybridization to a DNA of (a)-(d) under conditions of high stringency and which encodes polypeptides of the invention, and (f) DNA which is degenerate, as a result of the genetic code, to a DNA defined in (a), (b), (c), (d) or (e) and which encode polypeptides of the invention. DNA that encodes fragments of the polypeptide of SEQ ID NO:2 that are biologically active, e.g. bind an IL-1 receptor are also encompassed by the present invention. Of course, polypeptides encoded by such DNA sequences are encompassed by the invention.

As used herein, conditions of moderate stringency can be readily determined by those having ordinary skill in the art based on, for example, the length of the DNA. The basic conditions are set forth by Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2 ed. Vol. 1, pp. 1.101-104, Cold Spring Harbor Laboratory Press, (1989), and include use of a prewashing solution for the nitrocellulose filters 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization conditions of about 50% formamide, 6X SSC at about 42°C (or other similar hybridization solution, such as Stark's solution, in about 50% formamide at about 42°C), and washing conditions of about 60°C, 0.5X SSC, 0.1% SDS. Conditions of high stringency can also be readily determined by the skilled artisan based on, for example, the length of the DNA. Generally, such conditions are defined as hybridization conditions as above, and with washing at approximately 68°C, 0.2X SSC, 0.1% SDS. The skilled artisan will recognize that the temperature and wash solution salt concentration can be adjusted as necessary according to factors such as the length of the probe.

Also included encompassed by the invention is DNA encoding polypeptide fragments and DNA encoding polypeptides comprising inactivated N-glycosylation site(s), inactivated protease processing site(s), or conservative amino acid substitution(s), as described below.

In another embodiment, the polynucleotide molecules of the invention also include polynucleotides that are at least 80% identical to a native DNA. Also contemplated are embodiments in which a polynucleotide includes a molecules that is at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, or at least 99.9% identical to a native polynucleotide.

The percent identity may be determined by visual inspection and mathematical calculation. Alternatively, the percent identity of two polynucleotide sequences can be determined by comparing sequence information using the GAP computer program, version 6.0 described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res. 14*:6745, 1986, as described by Schwartz

and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps. Other programs used by one skilled in the art of sequence comparison may also be used.

The invention provides isolated polynucleotides useful in the production of polypeptides. Such polypeptides may be prepared by any of a number of conventional techniques. A DNA encoding a polypeptide of the invention, or desired fragment thereof may be subcloned into an expression vector for production of the polypeptide or fragment. The DNA advantageously is fused to a DNA encoding a suitable leader or signal peptide. Alternatively, the desired fragment may be chemically synthesized using known techniques. DNA fragments also may be produced by restriction endonuclease digestion of a full length cloned DNA, and isolated by electrophoresis on agarose gels. If necessary, oligonucleotides that reconstruct the 5' or 3' terminus to a desired point may be ligated to a DNA fragment generated by restriction enzyme digestion. Such oligonucleotides may additionally contain a restriction endonuclease cleavage site upstream of the desired coding sequence, and position an initiation codon (ATG) at the N-terminus of the coding sequence.

The well-known polymerase chain reaction (PCR) procedure also may be employed to isolate and amplify a DNA e encoding a desired protein fragment. Oligonucleotides that define the desired termini of the DNA fragment are employed as 5' and 3' primers. The oligonucleotides may additionally contain recognition sites for restriction endonucleases, to facilitate insertion of the amplified DNA fragment into an expression vector. PCR techniques are described in Saiki et al., *Science* 239:487 (1988); *Recombinant DNA Methodology*, Wu et al., eds., Academic Press, Inc., San Diego (1989), pp. 189-196; and *PCR Protocols: A Guide to Methods and Applications*, Innis et al., eds., Academic Press, Inc. (1990).

POLYPEPTIDES AND FRAGMENTS THEREOF

The invention encompasses polypeptides and fragments thereof in various forms, including those that are naturally occurring or produced through various techniques such as procedures involving recombinant DNA technology. Such forms include, but are not limited to, derivatives, variants, and oligomers, as well as fusion proteins or fragments thereof.

The polypeptides of the invention include full length proteins encoded by the polynucleotide sequences set forth above. Particularly preferred polypeptides of IL-1 eta comprise the amino acid sequence of SEQ ID NO:2.

The polypeptides of the invention may be secreted and, thus, soluble. Soluble polypeptides are capable of being secreted from the cells in which they are expressed. In general, soluble polypeptides may be identified (and distinguished from non-soluble membrane-bound counterparts) by separating intact cells which express the desired

polypeptide from the culture medium, *e.g.*, by centrifugation, and assaying the medium (supernatant) for the presence of the desired polypeptide. The presence of polypeptide in the medium indicates that the polypeptide was secreted from the cells and thus is a soluble form of the protein.

In one embodiment, the soluble polypeptides and fragments thereof comprise all or part of the extracellular domain, but lack the transmembrane region that would cause retention of the polypeptide on a cell membrane. A soluble polypeptide may include the cytoplasmic domain, or a portion thereof, as long as the polypeptide is secreted from the cell in which it is produced.

In general, the use of soluble forms is advantageous for certain applications. Purification of the polypeptides from recombinant host cells is facilitated, since the soluble polypeptides are secreted from the cells. Further, soluble polypeptides are generally more suitable for intravenous administration.

The invention also provides polypeptides and fragments of the extracellular domain that retain a desired biological activity. Particular embodiments are directed to polypeptide fragments of SEQ ID NO:2 that retain the ability to bind the native cognates, substrates, or counter-structure ("binding partner"). Such a fragment may be a soluble polypeptide, as described above. In another embodiment, the polypeptides and fragments advantageously include regions that are conserved in the IL-1 ligand and IL-1 receptor family as described above.

Also provided herein are polypeptide fragments comprising at least 20, or at least 30, contiguous amino acids of the sequences of SEQ ID NO:2. Polypeptide fragments also may be employed as immunogens, in generating antibodies.

Naturally occurring variants as well as derived variants of the polypeptides and fragments are provided herein.

Variants may exhibit amino acid sequences that are at least 80% identical. Also contemplated are embodiments in which a polypeptide or fragment comprises an amino acid sequence that is at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, or at least 99.9% identical to the preferred polypeptide or fragment thereof. Percent identity may be determined by visual inspection and mathematical calculation. Alternatively, the percent identity of two protein sequences can be determined by comparing sequence information using the GAP computer program, based on the algorithm of Needleman and Wunsch (J. Mol. Bio. 48:443, 1970) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The preferred default parameters for the GAP program include: (1) a scoring matrix, blosum62, as described by Henikoff and Henikoff (Proc. Natl. Acad. Sci. USA 89:10915, 1992); (2) a gap weight of 12; (3) a gap length weight of 4; and (4) no penalty for end gaps. Other programs used by one skilled in the art of sequence comparison may also be used.

The variants of the invention include, for example, those that result from alternate mRNA splicing events or from proteolytic cleavage. Alternate splicing of mRNA may, for example, yield a truncated but biologically active protein, such as a naturally occurring soluble form of the protein. Variations attributable to proteolysis include, for example, differences in the N- or C-termini upon expression in different types of host cells, due to proteolytic removal of one or more terminal amino acids from the protein (generally from 1-5 terminal amino acids). Proteins in which differences in amino acid sequence are attributable to genetic polymorphism (allelic variation among individuals producing the protein) are also contemplated herein.

Additional variants within the scope of the invention include polypeptides that may be modified to create derivatives thereof by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives may be prepared by linking the chemical moieties to functional groups on amino acid side chains or at the N-terminus or C-terminus of a polypeptide. Conjugates comprising diagnostic (detectable) or therapeutic agents attached thereto are contemplated herein, as discussed in more detail below.

Other derivatives include covalent or aggregative conjugates of the polypeptides with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. Examples of fusion proteins are discussed below in connection with oligomers. Further, fusion proteins can comprise peptides added to facilitate purification and identification. Such peptides include, for example, poly-His or the antigenic identification peptides described in U.S. Patent No. 5,011,912 and in Hopp et al., *Bio/Technology* 6:1204, 1988. One such peptide is the FLAG® peptide, Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys, which is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. A murine hybridoma designated 4E11 produces a monoclonal antibody that binds the FLAG® peptide in the presence of certain divalent metal cations, as described in U.S. Patent 5,011,912, hereby incorporated by reference. The 4E11 hybridoma cell line has been deposited with the American Type Culture Collection under accession no. HB 9259. Monoclonal antibodies that bind the FLAG® peptide are available from Eastman Kodak Co., Scientific Imaging Systems Division, New Haven, Connecticut.

Among the variant polypeptides provided herein are variants of native polypeptides that retain the native biological activity or the substantial equivalent thereof. One example is a variant that binds with essentially the same binding affinity as does the native form. Binding affinity can be measured by conventional procedures, *e.g.*, as described in U.S. Patent No. 5,512,457 and as set forth below.

Variants include polypeptides that are substantially homologous to the native form, but which have an amino acid sequence different from that of the native form because of one

or more deletions, insertions or substitutions. Particular embodiments include, but are not limited to, polypeptides that comprise from one to ten deletions, insertions or substitutions of amino acid residues, when compared to a native sequence.

A given amino acid may be replaced, for example, by a residue having similar physiochemical characteristics. Examples of such conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another; substitutions of one polar residue for another, such as between Lys and Arg, Glu and Asp, or Gln and Asn; or substitutions of one aromatic residue for another, such as Phe, Trp, or Tyr for one another. Other conservative substitutions, *e.g.*, involving substitutions of entire regions having similar hydrophobicity characteristics, are well known.

Similarly, the DNAs of the invention include variants that differ from a native DNA sequence because of one or more deletions, insertions or substitutions, but that encode a biologically active polypeptide.

The invention further includes polypeptides of the invention with or without associated native-pattern glycosylation. Polypeptides expressed in yeast or mammalian expression systems (*e.g.*, COS-1 or COS-7 cells) can be similar to or significantly different from a native polypeptide in molecular weight and glycosylation pattern, depending upon the choice of expression system. Expression of polypeptides of the invention in bacterial expression systems, such as *E. coli*, provides non-glycosylated molecules. Further, a given preparation may include multiple differentially glycosylated species of the protein. Glycosyl groups can be removed through conventional methods, in particular those utilizing glycopeptidase. In general, glycosylated polypeptides of the invention can be incubated with a molar excess of glycopeptidase (Boehringer Mannheim).

Correspondingly, similar DNA constructs that encode various additions or substitutions of amino acid residues or sequences, or deletions of terminal or internal residues or sequences are encompassed by the invention. For example, N-glycosylation sites in the polypeptide extracellular domain can be modified to preclude glycosylation, allowing expression of a reduced carbohydrate analog in mammalian and yeast expression systems. N-glycosylation sites in eukaryotic polypeptides are characterized by an amino acid triplet Asn-X-Y, wherein X is any amino acid except Pro and Y is Ser or Thr. Appropriate substitutions, additions, or deletions to the nucleotide sequence encoding these triplets will result in prevention of attachment of carbohydrate residues at the Asn side chain. Alteration of a single nucleotide, chosen so that Asn is replaced by a different amino acid, for example, is sufficient to inactivate an N-glycosylation site. Alternatively, the Ser or Thr can by replaced with another amino acid, such as Ala. Known procedures for inactivating N-glycosylation sites in proteins include those described in U.S. Patent 5,071,972 and EP 276,846, hereby incorporated by reference.

In another example of variants, nucleotides encoding Cys residues that are not essential for biological activity can be altered to cause the Cys residues to be deleted or replaced with other amino acids, preventing formation of incorrect intramolecular disulfide bridges upon folding or renaturation.

Other variants are prepared by modification of adjacent dibasic amino acid residues, to enhance expression in yeast systems in which KEX2 protease activity is present. EP 212,914 discloses the use of site-specific mutagenesis to inactivate KEX2 protease processing sites in a protein. KEX2 protease processing sites are inactivated by deleting, adding or substituting residues to alter Arg-Arg, Arg-Lys, and Lys-Arg pairs to eliminate the occurrence of these adjacent basic residues. Lys-Lys pairings are considerably less susceptible to KEX2 cleavage, and conversion of Arg-Lys or Lys-Arg to Lys-Lys represents a conservative and preferred approach to inactivating KEX2 sites.

Encompassed by the invention are oligomers or fusion proteins that contain IL-1 eta polypeptides. Such oligomers may be in the form of covalently-linked or non-covalently-linked multimers, including dimers, trimers, or higher oligomers. As noted above, preferred polypeptides are soluble and thus these oligomers may comprise soluble polypeptides. In one aspect of the invention, the oligomers maintain the binding ability of the polypeptide components and provide therefor, bivalent, trivalent, etc., binding sites.

One embodiment of the invention is directed to oligomers comprising multiple polypeptides joined *via* covalent or non-covalent interactions between peptide moieties fused to the polypeptides. Such peptides may be peptide linkers (spacers), or peptides that have the property of promoting oligomerization. Leucine zippers and certain polypeptides derived from antibodies are among the peptides that can promote oligomerization of the polypeptides attached thereto, as described in more detail below.

As one alternative, an oligomer is prepared using polypeptides derived from immunoglobulins. Preparation of fusion proteins comprising certain heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, *e.g.*, by Ashkenazi et al. (*PNAS USA* 88:10535, 1991); Byrn et al. (*Nature* 344:677, 1990); and Hollenbaugh and Aruffo ("Construction of Immunoglobulin Fusion Proteins", in *Current Protocols in Immunology*, Suppl. 4, pages 10.19.1 - 10.19.11, 1992).

One embodiment of the present invention is directed to a dimer comprising two fusion proteins created by fusing a polypeptide of the invention to an Fc polypeptide derived from an antibody. A gene fusion encoding the polypeptide/Fc fusion protein is inserted into an appropriate expression vector. Polypeptide/Fc fusion proteins are expressed in host cells transformed with the recombinant expression vector, and allowed to assemble much like antibody molecules, whereupon interchain disulfide bonds form between the Fc moieties to yield divalent molecules.

The term "Fc polypeptide" as used herein includes native and mutein forms of polypeptides made up of the Fc region of an antibody comprising any or all of the CH domains of the Fc region. Truncated forms of such polypeptides containing the hinge region that promotes dimerization are also included. Preferred polypeptides comprise an Fc polypeptide derived from a human IgG1 antibody.

One suitable Fc polypeptide, described in PCT application WO 93/10151, hereby incorporated by reference, is a single chain polypeptide extending from the N-terminal hinge region to the native C-terminus of the Fc region of a human IgG1 antibody. Another useful Fc polypeptide is the Fc mutein described in U.S. Patent 5,457,035 and in Baum et al., (*EMBO J.* 13:3992-4001, 1994) incorporated herein by reference. The amino acid sequence of this mutein is identical to that of the native Fc sequence presented in WO 93/10151, except that amino acid 19 has been changed from Leu to Ala, amino acid 20 has been changed from Leu to Glu, and amino acid 22 has been changed from Gly to Ala. The mutein exhibits reduced affinity for Fc receptors.

The above-described fusion proteins comprising Fc moieties (and oligomers formed therefrom) offer the advantage of facile purification by affinity chromatography over Protein A or Protein G columns.

In other embodiments, the polypeptides of the invention may be substituted for the variable portion of an antibody heavy or light chain. If fusion proteins are made with both heavy and light chains of an antibody, it is possible to form an oligomer with as many as four polypeptide extracellular regions.

Alternatively, the oligomer is a fusion protein comprising multiple polypeptides, with or without peptide linkers (spacer peptides). Among the suitable peptide linkers are those described in U.S. Patents 4,751,180 and 4,935,233, which are hereby incorporated by reference. A DNA sequence encoding a desired peptide linker may be inserted between, and in the same reading frame as, the DNA sequences of the invention, using any suitable conventional technique. For example, a chemically synthesized oligonucleotide encoding the linker may be ligated between the sequences. In particular embodiments, a fusion protein comprises from two to four soluble polypeptides of the invention, separated by peptide linkers.

Another method for preparing the oligomers of the invention involves use of a leucine zipper. Leucine zipper domains are peptides that promote oligomerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, 1988), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize.

The zipper domain (also referred to herein as an oligomerizing, or oligomer-forming, domain) comprises a repetitive heptad repeat, often with four or five leucine residues

interspersed with other amino acids. Examples of zipper domains are those found in the yeast transcription factor GCN4 and a heat-stable DNA-binding protein found in rat liver (C/EBP; Landschulz et al., *Science* 243:1681, 1989). Two nuclear transforming proteins, *fos* and *jun*, also exhibit zipper domains, as does the gene product of the murine proto-oncogene, c-myc (Landschulz et al., *Science* 240:1759, 1988). The products of the nuclear oncogenes *fos* and *jun* comprise zipper domains that preferentially form heterodimers (O'Shea et al., *Science* 245:646, 1989, Turner and Tjian, *Science* 243:1689, 1989). The zipper domain is necessary for biological activity (DNA binding) in these proteins.

The fusogenic proteins of several different viruses, including paramyxovirus, coronavirus, measles virus and many retroviruses, also possess zipper domains (Buckland and Wild, *Nature* 338:547,1989; Britton, *Nature* 353:394, 1991; Delwart and Mosialos, *AIDS Research and Human Retroviruses* 6:703, 1990). The zipper domains in these fusogenic viral proteins are near the transmembrane region of the proteins; it has been suggested that the zipper domains could contribute to the oligomeric structure of the fusogenic proteins. Oligomerization of fusogenic viral proteins is involved in fusion pore formation (Spruce et al, *Proc. Natl. Acad. Sci. U.S.A.* 88:3523, 1991). Zipper domains have also been recently reported to play a role in oligomerization of heat-shock transcription factors (Rabindran et al., *Science* 259:230, 1993).

Zipper domains fold as short, parallel coiled coils. (O'Shea et al., *Science* 254:539; 1991) The general architecture of the parallel coiled coil has been well characterized, with a "knobs-into-holes" packing as proposed by Crick in 1953 (*Acta Crystallogr.* 6:689). The dimer formed by a zipper domain is stabilized by the heptad repeat, designated $(abcdefg)_n$ according to the notation of McLachlan and Stewart (*J. Mol. Biol.* 98:293; 1975), in which residues a and d are generally hydrophobic residues, with d being a leucine, which line up on the same face of a helix. Oppositely-charged residues commonly occur at positions g and e. Thus, in a parallel coiled coil formed from two helical zipper domains, the "knobs" formed by the hydrophobic side chains of the first helix are packed into the "holes" formed between the side chains of the second helix.

The residues at position d (often leucine) contribute large hydrophobic stabilization energies, and are important for oligomer formation (Krystek: et al., *Int. J. Peptide Res.* 38:229, 1991). Lovejoy et al. (*Science* 259:1288, 1993) recently reported the synthesis of a triple-stranded α-helical bundle in which the helices run up-up-down. Their studies confirmed that hydrophobic stabilization energy provides the main driving force for the formation of coiled coils from helical monomers. These studies also indicate that electrostatic interactions contribute to the stoichiometry and geometry of coiled coils. Further discussion of the structure of leucine zippers is found in Harbury et al. (*Science* 262:1401, 26 November 1993)

Examples of leucine zipper domains suitable for producing soluble oligomeric proteins are described in PCT application WO 94/10308, and the leucine zipper derived from lung surfactant protein D (SPD) described in Hoppe et al. (*FEBS Letters* 344:191, 1994), hereby incorporated by reference. The use of a modified leucine zipper that allows for stable trimerization of a heterologous protein fused thereto is described in Fanslow et al. (*Semin. Immunol.* 6:267-278, 1994). Recombinant fusion proteins comprising a soluble polypeptide fused to a leucine zipper peptide are expressed in suitable host cells, and the soluble oligomer that forms is recovered from the culture supernatant.

Certain leucine zipper moieties preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (*FEBS Letters* 344:191, 1994) and in U.S. Patent 5,716,805, hereby incorporated by reference in their entirety. This lung SPD-derived leucine zipper peptide comprises the amino acid sequence Pro Asp Val Ala Ser Leu Arg Gln Gln Val Glu Ala Leu Gln Gly Gln Val Gln His Leu Gln Ala Ala Phe Ser Gln Tyr.

Another example of a leucine zipper that promotes trimerization is a peptide comprising the amino acid sequence Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser Lys Ile Tyr His Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu Arg, as described in U.S. Patent 5,716,805. In one alternative embodiment, an N-terminal Asp residue is added; in another, the peptide lacks the N-terminal Arg residue.

Fragments of the foregoing zipper peptides that retain the property of promoting oligomerization may be employed as well. Examples of such fragments include, but are not limited to, peptides lacking one or two of the N-terminal or C-terminal residues presented in the foregoing amino acid sequences. Leucine zippers may be derived from naturally occurring leucine zipper peptides, e.g., *via* conservative substitution(s) in the native amino acid sequence, wherein the peptide's ability to promote oligomerization is retained.

Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric oligomers. Alternatively, synthetic peptides that promote oligomerization may be employed. In particular embodiments, leucine residues in a leucine zipper moiety are replaced by isoleucine residues. Such peptides comprising isoleucine may be referred to as isoleucine zippers, but are encompassed by the term "leucine zippers" as employed herein.

PRODUCTION OF POLYPEPTIDES AND FRAGMENTS THEREOF

Expression, isolation and purification of the polypeptides and fragments of the invention may be accomplished by any suitable technique, including but not limited to the following:

The present invention also provides recombinant cloning and expression vectors containing DNA. as well as host cell containing the recombinant vectors. Expression vectors comprising DNA may be used to prepare the polypeptides or fragments of the invention encoded by the DNA. A method for producing polypeptides comprises culturing host cells transformed with a recombinant expression vector encoding the polypeptide, under conditions that promote expression of the polypeptide, then recovering the expressed polypeptides from the culture. The skilled artisan will recognize that the procedure for purifying the expressed polypeptides will vary according to such factors as the type of host cells employed, and whether the polypeptide is membrane-bound or a soluble form that is secreted from the host cell.

Any suitable expression system may be employed. The vectors include a DNA encoding a polypeptide or fragment of the invention, operably linked to suitable transcriptional or translational regulatory nucleotide sequences, such as those derived from a mammalian, microbial, viral, or insect gene. Examples of regulatory sequences include transcriptional promoters, operators, or enhancers, an mRNA ribosomal binding site, and appropriate sequences which control transcription and translation initiation and termination. Nucleotide sequences are operably linked when the regulatory sequence functionally relates to the DNA sequence. Thus, a promoter nucleotide sequence is operably linked to a DNA sequence if the promoter nucleotide sequence controls the transcription of the DNA sequence. An origin of replication that confers the ability to replicate in the desired host cells, and a selection gene by which transformants are identified, are generally incorporated into the expression vector.

In addition, a sequence encoding an appropriate signal peptide (native or heterologous) can be incorporated into expression vectors. A DNA sequence for a signal peptide (secretory leader) may be fused in frame to the polynucleotide sequence of the invention so that the DNA is initially transcribed, and the mRNA translated, into a fusion protein comprising the signal peptide. A signal peptide that is functional in the intended host cells promotes extracellular secretion of the polypeptide. The signal peptide is cleaved from the polypeptide upon secretion of polypeptide from the cell.

The skilled artisan will also recognize that the position(s) at which the signal peptide is cleaved may differ from that predicted by computer program, and may vary according to such factors as the type of host cells employed in expressing a recombinant polypeptide. A protein preparation may include a mixture of protein molecules having different N-terminal amino acids, resulting from cleavage of the signal peptide at more than one site.

Suitable host cells for expression of polypeptides include prokaryotes, yeast or higher eukaryotic cells. Mammalian or insect cells are generally preferred for use as host cells. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described, for example, in Pouwels et al. *Cloning Vectors: A*

Laboratory Manual, Elsevier, New York, (1985). Cell-free translation systems could also be employed to produce polypeptides using RNAs derived from DNA constructs disclosed herein.

Prokaryotes include gram-negative or gram-positive organisms. Suitable prokaryotic host cells for transformation include, for example, *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various other species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*. In a prokaryotic host cell, such as *E. coli*, a polypeptide may include an N-terminal methionine residue to facilitate expression of the recombinant polypeptide in the prokaryotic host cell. The N-terminal Met may be cleaved from the expressed recombinant polypeptide.

Expression vectors for use in prokaryotic host cells generally comprise one or more phenotypic selectable marker genes. A phenotypic selectable marker gene is, for example, a gene encoding a protein that confers antibiotic resistance or that supplies an autotrophic requirement. Examples of useful expression vectors for prokaryotic host cells include those derived from commercially available plasmids such as the cloning vector pBR322 (ATCC 37017). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells. An appropriate promoter and a DNA sequence are inserted into the pBR322 vector. Other commercially available vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA).

Promoter sequences commonly used for recombinant prokaryotic host cell expression vectors include β-lactamase (penicillinase), lactose promoter system (Chang et al., *Nature 275*:615, 1978; and Goeddel et al., *Nature 281*:544, 1979), tryptophan (trp) promoter system (Goeddel et al., *Nucl. Acids Res. 8*:4057, 1980; and EP-A-36776) and tac promoter (Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982). A particularly useful prokaryotic host cell expression system employs a phage λP_L promoter and a cI857ts thermolabile repressor sequence. Plasmid vectors available from the American Type Culture Collection which incorporate derivatives of the λP_L promoter include plasmid pHUB2 (resident in *E. coli* strain JMB9, ATCC 37092) and pPLc28 (resident in *E. coli* RR1, ATCC 53082).

Alternatively, the polypeptides may be expressed in yeast host cells, preferably from the *Saccharomyces* genus (*e.g.*, *S. cerevisiae*). Other genera of yeast, such as *Pichia* or *Kluyveromyces*, may also be employed. Yeast vectors will often contain an origin of replication sequence from a 2μ yeast plasmid, an autonomously replicating sequence (ARS), a promoter region, sequences for polyadenylation, sequences for transcription termination, and a selectable marker gene. Suitable promoter sequences for yeast vectors include, among others, promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem. 255*:2073, 1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg. 7*:149,

1968; and Holland et al., *Biochem.* *17*:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phospho-glucose isomerase, and glucokinase. Other suitable vectors and promoters for use in yeast expression are further described in Hitzeman, EPA-73,657. Another alternative is the glucose-repressible ADH2 promoter described by Russell et al. (*J. Biol. Chem.* *258*:2674, 1982) and Beier et al. (*Nature* *300*:724, 1982). Shuttle vectors replicable in both yeast and *E. coli* may be constructed by inserting DNA sequences from pBR322 for selection and replication in *E. coli* (Amp^r gene and origin of replication) into the above-described yeast vectors.

The yeast α-factor leader sequence may be employed to direct secretion of the polypeptide. The α-factor leader sequence is often inserted between the promoter sequence and the structural gene sequence. *See*, e.g., Kurjan et al., *Cell* *30*:933, 1982 and Bitter et al., *Proc. Natl. Acad. Sci. USA* *81*:5330, 1984. Other leader sequences suitable for facilitating secretion of recombinant polypeptides from yeast hosts are known to those of skill in the art. A leader sequence may be modified near its 3' end to contain one or more restriction sites. This will facilitate fusion of the leader sequence to the structural gene.

Yeast transformation protocols are known to those of skill in the art. One such protocol is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA* *75*:1929, 1978. The Hinnen et al. protocol selects for Trp^+ transformants in a selective medium, wherein the selective medium consists of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 mg/ml adenine and 20 mg/ml uracil.

Yeast host cells transformed by vectors containing an ADH2 promoter sequence may be grown for inducing expression in a "rich" medium. An example of a rich medium is one consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 mg/ml adenine and 80 mg/ml uracil. Derepression of the ADH2 promoter occurs when glucose is exhausted from the medium.

Mammalian or insect host cell culture systems also may be employed to express recombinant polypeptides. Bacculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988). Established cell lines of mammalian origin also may be employed. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman et al., *Cell* *23*:175, 1981), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells, HeLa cells, and BHK (ATCC CRL 10) cell lines, and the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) as described by McMahan et al. (*EMBO J.* 10: 2821, 1991).

Established methods for introducing DNA into mammalian cells have been described (Kaufman, R.J., *Large Scale Mammalian Cell Culture*, 1990, pp. 15-69). Additional

protocols using commercially available reagents, such as Lipofectamine lipid reagent (Gibco/BRL) or Lipofectamine-Plus lipid reagent, can be used to transfect cells (Felgner et al., *Proc. Natl. Acad. Sci. USA 84*:7413-7417, 1987). In addition, electroporation can be used to transfect mammalian cells using conventional procedures, such as those in Sambrook et al. (*Molecular Cloning: A Laboratory Manual*, 2 ed. Vol. 1-3, Cold Spring Harbor Laboratory Press, 1989). Selection of stable transformants can be performed using methods known in the art, such as, for example, resistance to cytotoxic drugs. Kaufman et al., *Meth. in Enzymology 185*:487-511, 1990, describes several selection schemes, such as dihydrofolate reductase (DHFR) resistance. A suitable host strain for DHFR selection can be CHO strain DX-B11, which is deficient in DHFR (Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA 77*:4216-4220, 1980). A plasmid expressing the DHFR cDNA can be introduced into strain DX-B11, and only cells that contain the plasmid can grow in the appropriate selective media. Other examples of selectable markers that can be incorporated into an expression vector include cDNAs conferring resistance to antibiotics, such as G418 and hygromycin B. Cells harboring the vector can be selected on the basis of resistance to these compounds.

Transcriptional and translational control sequences for mammalian host cell expression vectors can be excised from viral genomes. Commonly used promoter sequences and enhancer sequences are derived from polyoma virus, adenovirus 2, simian virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites can be used to provide other genetic elements for expression of a structural gene sequence in a mammalian host cell. Viral early and late promoters are particularly useful because both are easily obtained from a viral genome as a fragment, which can also contain a viral origin of replication (Fiers et al., *Nature 273*:113, 1978; Kaufman, *Meth. in Enzymology*, 1990). Smaller or larger SV40 fragments can also be used, provided the approximately 250 bp sequence extending from the *Hind* III site toward the *Bgl* I site located in the SV40 viral origin of replication site is included.

Additional control sequences shown to improve expression of heterologous genes from mammalian expression vectors include such elements as the expression augmenting sequence element (EASE) derived from CHO cells (Morris et al., *Animal Cell Technology*, 1997, pp. 529-534 and PCT Application WO 97/25420) and the tripartite leader (TPL) and VA gene RNAs from Adenovirus 2 (Gingeras et al., *J. Biol. Chem. 257*:13475-13491, 1982). The internal ribosome entry site (IRES) sequences of viral origin allows dicistronic mRNAs to be translated efficiently (Oh and Sarnow, *Current Opinion in Genetics and Development 3*:295-300, 1993; Ramesh et al., *Polynucleotides Research 24*:2697-2700, 1996). Expression of a heterologous cDNA as part of a dicistronic mRNA followed by the gene for a selectable marker (e.g. DHFR) has been shown to improve transfectability of the host and expression of the heterologous cDNA (Kaufman, *Meth. in Enzymology*, 1990). Exemplary expression

vectors that employ dicistronic mRNAs are pTR-DC/GFP described by Mosser et al., *Biotechniques* 22:150-161, 1997, and p2A5I described by Morris et al., *Animal Cell Technology*, 1997, pp. 529-534.

A useful high expression vector, pCAVNOT, has been described by Mosley et al., *Cell 59*:335-348, 1989. Other expression vectors for use in mammalian host cells can be constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol. 3*:280, 1983). A useful system for stable high level expression of mammalian cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol. 23*:935, 1986). A useful high expression vector, PMLSV N1/N4, described by Cosman et al., *Nature 312*:768, 1984, has been deposited as ATCC 39890. Additional useful mammalian expression vectors are described in EP-A-0367566, and in WO 91/18982, incorporated by reference herein. In yet another alternative, the vectors can be derived from retroviruses.

Another useful expression vector, pFLAG®, can be used. FLAG® technology is centered on the fusion of a low molecular weight (1kD), hydrophilic, FLAG® marker peptide to the N-terminus of a recombinant protein expressed by pFLAG® expression vectors.

Regarding signal peptides that may be employed, the native signal peptide may be replaced by a heterologous signal peptide or leader sequence, if desired. The choice of signal peptide or leader may depend on factors such as the type of host cells in which the recombinant polypeptide is to be produced. To illustrate, examples of heterologous signal peptides that are functional in mammalian host cells include the signal sequence for interleukin-7 (IL-7) described in United States Patent 4,965,195; the signal sequence for interleukin-2 receptor described in Cosman et al., *Nature* 312:768 (1984); the interleukin-4 receptor signal peptide described in EP 367,566; the type I interleukin-1 receptor signal peptide described in U.S. Patent 4,968,607; and the type II interleukin-1 receptor signal peptide described in EP 460,846.

The invention also includes methods of isolating and purifying the polypeptides and fragments thereof.

The "isolated" polypeptides or fragments thereof encompassed by this invention are polypeptides or fragments that are not in an environment identical to an environment in which it or they can be found in nature. The "purified" polypeptides or fragments thereof encompassed by this invention are essentially free of association with other proteins or polypeptides, for example, as a purification product of recombinant expression systems such as those described above or as a purified product from a non-recombinant source such as naturally occurring cells and/or tissues.

In one preferred embodiment, the purification of recombinant polypeptides or fragments can be accomplished using fusions of polypeptides or fragments of the invention to another polypeptide to aid in the purification of polypeptides or fragments of the invention.

Such fusion partners can include the poly-His or other antigenic identification peptides described above as well as the Fc moieties described previously.

With respect to any type of host cell, as is known to the skilled artisan, procedures for purifying a recombinant polypeptide or fragment will vary according to such factors as the type of host cells employed and whether or not the recombinant polypeptide or fragment is secreted into the culture medium.

In general, the recombinant polypeptide or fragment can be isolated from the host cells if not secreted, or from the medium or supernatant if soluble and secreted, followed by one or more concentration, salting-out, ion exchange, hydrophobic interaction, affinity purification or size exclusion chromatography steps. As to specific ways to accomplish these steps, the culture medium first can be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration medium. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. In addition, a chromatofocusing step can be employed. Alternatively, a hydrophobic interaction chromatography step can be employed. Suitable matrices can be phenyl or octyl moieties bound to resins. In addition, affinity chromatography with a matrix which selectively binds the recombinant protein can be employed. Examples of such resins employed are lectin columns, dye columns, and metal-chelating columns. Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, (e.g., silica gel or polymer resin having pendant methyl, octyl, octyldecyl or other aliphatic groups) can be employed to further purify the polypeptides. Some or all of the foregoing purification steps, in various combinations, are well known and can be employed to provide an isolated and purified recombinant protein.

It is also possible to utilize an affinity column comprising a polypeptide-binding protein of the invention, such as a monoclonal antibody generated against polypeptides of the invention, to affinity-purify expressed polypeptides. These polypeptides can be removed from an affinity column using conventional techniques, e.g., in a high salt elution buffer and then dialyzed into a lower salt buffer for use or by changing pH or other components depending on the affinity matrix utilized, or be competitively removed using the naturally occurring substrate of the affinity moiety, such as a polypeptide derived from the invention.

In this aspect of the invention, polypeptide-binding proteins, such as the anti-polypeptide antibodies of the invention or other proteins that may interact with the polypeptide of the invention, can be bound to a solid phase support such as a column

chromatography matrix or a similar substrate suitable for identifying, separating, or purifying cells that express polypeptides of the invention on their surface. Adherence of polypeptide-binding proteins of the invention to a solid phase contacting surface can be accomplished by any means. For example, magnetic microspheres can be coated with these polypeptide-binding proteins and held in the incubation vessel through a magnetic field. Suspensions of cell mixtures are contacted with the solid phase that has such polypeptide-binding proteins thereon. Cells having polypeptides of the invention on their surface bind to the fixed polypeptide-binding protein and unbound cells then are washed away. This affinity-binding method is useful for purifying, screening, or separating such polypeptide-expressing cells from solution. Methods of releasing positively selected cells from the solid phase are known in the art and encompass, for example, the use of enzymes. Such enzymes are preferably non-toxic and non-injurious to the cells and are preferably directed to cleaving the cell-surface binding partner.

Alternatively, mixtures of cells suspected of containing polypeptide-expressing cells of the invention first can be incubated with a biotinylated polypeptide-binding protein of the invention. Incubation periods are typically at least one hour in duration to ensure sufficient binding to polypeptides of the invention. The resulting mixture then is passed through a column packed with avidin-coated beads, whereby the high affinity of biotin for avidin provides the binding of the polypeptide-binding cells to the beads. Use of avidin-coated beads is known in the art. See Berenson, et al. *J. Cell. Biochem.*, 10D:239 (1986). Wash of unbound material and the release of the bound cells is performed using conventional methods.

The desired degree of purity depends on the intended use of the protein. A relatively high degree of purity is desired when the polypeptide is to be administered *in vivo*, for example. In such a case, the polypeptides are purified such that no protein bands corresponding to other proteins are detectable upon analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). It will be recognized by one skilled in the pertinent field that multiple bands corresponding to the polypeptide may be visualized by SDS-PAGE, due to differential glycosylation, differential post-translational processing, and the like. Most preferably, the polypeptide of the invention is purified to substantial homogeneity, as indicated by a single protein band upon analysis by SDS-PAGE. The protein band may be visualized by silver staining, Coomassie blue staining, or (if the protein is radiolabeled) by autoradiography.

The purified polypeptides of the invention (including proteins, polypeptides, fragments, variants, oligomers, and other forms) may be tested for the ability to bind the binding partner in any suitable assay, such as a conventional binding assay. To illustrate, the polypeptide may be labeled with a detectable reagent (e.g., a radionuclide, chromophore, enzyme that catalyzes a colorimetric or fluorometric reaction, and the like). The labeled

polypeptide is contacted with cells expressing the binding partner. The cells then are washed to remove unbound labeled polypeptide, and the presence of cell-bound label is determined by a suitable technique, chosen according to the nature of the label.

One example of a binding assay procedure is as follows. A recombinant expression vector containing the binding partner cDNA is constructed using methods well known in the art. CV1-EBNA-1 cells in 10 cm^2 dishes are transfected with the recombinant expression vector. CV-1/EBNA-1 cells (ATCC CRL 10478) constitutively express EBV nuclear antigen-1 driven from the CMV immediate-early enhancer/promoter. CV1-EBNA-1 was derived from the African Green Monkey kidney cell line CV-1 (ATCC CCL 70), as described by McMahan et al. (*EMBO J.* 10:2821, 1991).

The transfected cells are cultured for 24 hours, and the cells in each dish then are split into a 24-well plate. After culturing an additional 48 hours, the transfected cells (about 4 x 10^4 cells/well) are washed with BM-NFDM, which is binding medium (RPMI 1640 containing 25 mg/ml bovine serum albumin, 2 mg/ml sodium azide, 20 mM Hepes pH 7.2) to which 50 mg/ml nonfat dry milk has been added. The cells then are incubated for 1 hour at 37°C with various concentrations of, for example, a soluble polypeptide/Fc fusion protein made as set forth above. Cells then are washed and incubated with a constant saturating concentration of a ^{125}I-mouse anti-human IgG in binding medium, with gentle agitation for 1 hour at 37°C. After extensive washing, cells are released *via* trypsinization.

The mouse anti-human IgG employed above is directed against the Fc region of human IgG and can be obtained from Jackson Immunoresearch Laboratories, Inc., West Grove, PA. The antibody is radioiodinated using the standard chloramine-T method. The antibody will bind to the Fc portion of any polypeptide/Fc protein that has bound to the cells. In all assays, non-specific binding of ^{125}I-antibody is assayed in the absence of the Fc fusion protein/Fc, as well as in the presence of the Fc fusion protein and a 200-fold molar excess of unlabeled mouse anti-human IgG antibody.

Cell-bound ^{125}I-antibody is quantified on a Packard Autogamma counter. Affinity calculations (Scatchard, *Ann. N.Y. Acad. Sci.* 51:660, 1949) are generated on RS/1 (BBN Software, Boston, MA) run on a Microvax computer.

Another type of suitable binding assay is a competitive binding assay. To illustrate, biological activity of a variant may be determined by assaying for the variant's ability to compete with the native protein for binding to the binding partner.

Competitive binding assays can be performed by conventional methodology. Reagents that may be employed in competitive binding assays include radiolabeled polypeptides of the invention and intact cells expressing the binding partner (endogenous or recombinant). For example, a radiolabeled soluble IL-1 eta fragment can be used to compete with a soluble IL-1 eta variant for binding to cell surface IL-1 eta receptors. Instead of intact cells, one could substitute a soluble binding partner/Fc fusion protein bound to a solid phase

through the interaction of Protein A or Protein G (on the solid phase) with the Fc moiety. Chromatography columns that contain Protein A and Protein G include those available from Pharmacia Biotech, Inc., Piscataway, NJ.

Another type of competitive binding assay utilizes radiolabeled soluble binding partner, such as a soluble IL-1 eta receptor/Fc fusion protein, and intact cells expressing the binding partner. Qualitative results can be obtained by competitive autoradiographic plate binding assays, while Scatchard plots (Scatchard, *Ann. N.Y. Acad. Sci.* 51:660, 1949) may be utilized to generate quantitative results.

The IL-1 eta polypeptide of the present invention may also be used as a screening assay for compounds and small molecules which inhibit activation of (antagonists) the IL-1 eta polypeptide of the instant invention. Thus, polypeptides of the invention may be used to identify antagonists from, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. The antagonists may be natural or modified substrates, ligands, enzymes, receptors, etc. of the IL-1 eta polypeptide, or may be structural or functional mimetics of the IL-1 eta polypeptide. The antagonists may further be small molecules, peptides, antibodies and antisense oligonucleotides.

One embodiment of a method for identifying compounds which antagonize the IL-1 eta polypeptide is contacting a candidate compound with cells which respond to IL-1 eta polypeptide and observe the binding, or stimulation or inhibition of a functional response. The activity of the cells which were contacted with the candidate compound could then be compared with the identical cells which were not contacted for IL-1 eta polypeptide activity and IL-1 eta polypeptide agonists and antagonists could be identified. A still further embodiment of the instant invention provides a method of identifying compounds that inhibit the synthesis or secretion of IL-1 eta by contacting the candidate compound with cells which express IL-1 eta polypeptide and measuring the IL-1 eta production. The measurement of IL-1 eta production could be performed by a number of well-known methods such as measuring the amount of protein present (e.g. an ELISA) or of the protein's activity.

USE OF IL-1 ETA POLYNUCLEOTIDE OR OLIGONUCLEOTIDES

Among the uses of polynucleotides of the invention is the use of polynucleotide fragments or oligonucleotides as probes or primers. Such fragments generally comprise at least about 17 contiguous nucleotides of a DNA sequence. In other embodiments, a DNA fragment comprises at least 30, or at least 60, contiguous nucleotides of a DNA sequence.

Because homologs of SEQ ID NO:1, from other mammalian species, are contemplated herein, probes based on the human DNA sequence of SEQ ID NO:1 may be used to screen cDNA libraries derived from other mammalian species, using conventional cross-species hybridization techniques.

Using knowledge of the genetic code in combination with the amino acid sequences set forth above, sets of degenerate oligonucleotides can be prepared. Such oligonucleotides are useful as primers, e.g., in polymerase chain reactions (PCR), whereby DNA fragments are isolated and amplified.

All or a portion of the polynucleotides of IL-1 eta of SEQ ID NO:1, including oligonucleotides, can be used by those skilled in the art using well-known techniques to identify the human chromosome 2, as well as the specific locus thereof, that contains the DNA of IL-1 ligand family members. Useful techniques include, but are not limited to, using the sequence or portions, including oligonucleotides, as a probe in various well-known techniques such as radiation hybrid mapping (high resolution), in situ hybridization to chromosome spreads (moderate resolution), and Southern blot hybridization to hybrid cell lines containing individual human chromosomes (low resolution).

For example, chromosomes can be mapped by radiation hybridization which involves using PCR and the Whitehead Institute/MIT Center for Genome Research Genebridge4 panel of 93 radiation hybrids (http://www-genome.wi.mit.edu/ftp/distribution/human_STS_releases/july97/rhmap/genebridge4.html). The PCR primers lie within the gene of interest and amplify a product from human genomic DNA, but do not amplify hamster genomic DNA. The results of the PCRs are converted into a data vector that is submitted to the Whitehead/MIT Radiation Mapping site on the internet (http://www-seq.wi.mit.edu). The data is scored and the chromosomal assignment and placement relative to known Sequence Tag Site (STS) markers on the radiation hybrid map is provided. The following web site provides additional information about radiation hybrid mapping: http://www-genome.wi.mit.edu/ftp/distribution/human_STS_releases/july97/07-97.INTRO.html).

The DNA SEQ ID NO:1 has been mapped by radiation hybridization to the 2q11-12 region of human chromosome 2. Human chromosome 2 is associated with specific diseases which include but are not limited to glaucoma, ectodermal dysplasia, insulin-dependent diabetes mellitus, wrinkly skin syndrome, T-cell leukemia/lymphoma, and tibial muscular dystrophy. Thus, the polynucleotides of SEQ ID NO:1 or a fragment thereof can be used by one skilled in the art using well-known techniques to analyze abnormalities associated with gene mapping to chromosome 2. This enables one to distinguish conditions in which this marker is rearranged or deleted. In addition, nucleotides of SEQ ID NO:1 or a fragment thereof can be used as a positional marker to map other genes of unknown location.

The DNA may be used in developing treatments for any disorder mediated (directly or indirectly) by defective, or insufficient amounts of, the genes corresponding to the polynucleotides of the invention. Disclosure herein of native nucleotide sequences permits the detection of defective genes, and the replacement thereof with normal genes. Defective genes may be detected in *in vitro* diagnostic assays, and by comparison of a native nucleotide

sequence disclosed herein with that of a gene derived from a person suspected of harboring a defect in this gene.

Other useful fragments of the polynucleotides include antisense or sense oligonucleotides comprising a single-stranded polynucleotide sequence (either RNA or DNA) capable of binding to target mRNA (sense) or DNA (antisense) sequences. Antisense or sense oligonucleotides according to the present invention comprise a fragment of DNA (SEQ ID NO:1). Such a fragment generally comprises at least about 14 nucleotides, preferably from about 14 to about 30 nucleotides. The ability to derive an antisense or a sense oligonucleotide, based upon a cDNA sequence encoding a given protein is described in, for example, Stein and Cohen (*Cancer Res.* 48:2659, 1988) and van der Krol et al. (*BioTechniques* 6:958, 1988).

Binding of antisense or sense oligonucleotides to target polynucleotide sequences results in the formation of duplexes that block or inhibit protein expression by one of several means, including enhanced degradation of the mRNA by RNAseH, inhibition of splicing, premature termination of transcription or translation, or by other means. The antisense oligonucleotides thus may be used to block expression of proteins. Antisense or sense oligonucleotides further comprise oligonucleotides having modified sugar-phosphodiester backbones (or other sugar linkages, such as those described in WO91/06629) and wherein such sugar linkages are resistant to endogenous nucleases. Such oligonucleotides with resistant sugar linkages are stable *in vivo* (*i.e.*, capable of resisting enzymatic degradation) but retain sequence specificity to be able to bind to target nucleotide sequences.

Other examples of sense or antisense oligonucleotides include those oligonucleotides which are covalently linked to organic moieties, such as those described in WO 90/10448, and other moieties that increases affinity of the oligonucleotide for a target polynucleotide sequence, such as poly-(L-lysine). Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or antisense oligonucleotides to modify binding specificities of the antisense or sense oligonucleotide for the target nucleotide sequence.

Antisense or sense oligonucleotides may be introduced into a cell containing the target polynucleotide sequence by any gene transfer method, including, for example, lipofection, $CaPO_4$-mediated DNA transfection, electroporation, or by using gene transfer vectors such as Epstein-Barr virus.

Sense or antisense oligonucleotides also may be introduced into a cell containing the target nucleotide sequence by formation of a conjugate with a ligand binding molecule, as described in WO 91/04753. Suitable ligand binding molecules include, but are not limited to, cell surface receptors, growth factors, other cytokines, or other ligands that bind to cell surface receptors. Preferably, conjugation of the ligand binding molecule does not substantially interfere with the ability of the ligand binding molecule to bind to its

corresponding molecule or receptor, or block entry of the sense or antisense oligonucleotide or its conjugated version into the cell.

Alternatively, a sense or an antisense oligonucleotide may be introduced into a cell containing the target polynucleotide sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. The sense or antisense oligonucleotide-lipid complex is preferably dissociated within the cell by an endogenous lipase.

USE OF IL-1 ETA POLYPEPTIDES AND FRAGMENTED POLYPEPTIDES

Each of the polypeptides of the invention finds use as a protein purification reagent. The polypeptides may be attached to a solid support material and used to purify the binding partner proteins by affinity chromatography. In particular embodiments, a polypeptide (in any form described herein that is capable of binding the binding partner) is attached to a solid support by conventional procedures. As one example, chromatography columns containing functional groups that will react with functional groups on amino acid side chains of proteins are available (Pharmacia Biotech, Inc., Piscataway, NJ). In an alternative, a polypeptide/Fc protein (as discussed above) is attached to Protein A- or Protein G-containing chromatography columns through interaction with the Fc moiety.

The polypeptide also finds use in purifying or identifying cells that express the binding partner on the cell surface. Polypeptides are bound to a solid phase such as a column chromatography matrix or a similar suitable substrate. For example, magnetic microspheres can be coated with the polypeptides and held in an incubation vessel through a magnetic field. Suspensions of cell mixtures containing the binding partner expressing cells are contacted with the solid phase having the polypeptides thereon. Cells expressing the binding partner on the cell surface bind to the fixed polypeptides, and unbound cells then are washed away.

Alternatively, the polypeptides can be conjugated to a detectable moiety, then incubated with cells to be tested for binding partner expression. After incubation, unbound labeled matter is removed and the presence or absence of the detectable moiety on the cells is determined.

In a further alternative, mixtures of cells suspected of containing cells expressing the binding partner are incubated with biotinylated polypeptides. Incubation periods are typically at least one hour in duration to ensure sufficient binding. The resulting mixture then is passed through a column packed with avidin-coated beads, whereby the high affinity of biotin for avidin provides binding of the desired cells to the beads. Procedures for using avidin-coated beads are known (see Berenson, et al. *J. Cell. Biochem.*, 10D:239, 1986). Washing to remove unbound material, and the release of the bound cells, are performed using conventional methods.

Polypeptides also find use in measuring the biological activity of the binding partner protein in terms of their binding affinity. The polypeptides thus may be employed by those conducting "quality assurance" studies, e.g., to monitor shelf life and stability of protein under different conditions. For example, the polypeptides may be employed in a binding affinity study to measure the biological activity of a binding partner protein that has been stored at different temperatures, or produced in different cell types. The proteins also may be used to determine whether biological activity is retained after modification of a binding partner protein (e.g., chemical modification, truncation, mutation, etc.). The binding affinity of the modified binding partner protein is compared to that of an unmodified binding partner protein to detect any adverse impact of the modifications on biological activity of the binding partner. The biological activity of a binding partner protein thus can be ascertained before it is used in a research study, for example.

The polypeptides also find use as carriers for delivering agents attached thereto to cells bearing the binding partner. The polypeptides thus can be used to deliver diagnostic or therapeutic agents to such cells (or to other cell types found to express the binding partner on the cell surface) in *in vitro* or *in vivo* procedures.

Detectable (diagnostic) and therapeutic agents that may be attached to a polypeptide include, but are not limited to, toxins, other cytotoxic agents, drugs, radionuclides, chromophores, enzymes that catalyze a colorimetric or fluorometric reaction, and the like, with the particular agent being chosen according to the intended application. Among the toxins are ricin, abrin, diphtheria toxin, *Pseudomonas aeruginosa* exotoxin A, ribosomal inactivating proteins, mycotoxins such as trichothecenes, and derivatives and fragments (e.g., single chains) thereof. Radionuclides suitable for diagnostic use include, but are not limited to, ^{123}I, ^{131}I, ^{99m}Tc, ^{111}In, and ^{76}Br. Examples of radionuclides suitable for therapeutic use are ^{131}I, ^{211}At, ^{77}Br, ^{186}Re, ^{188}Re, ^{212}Pb, ^{212}Bi, ^{109}Pd, ^{64}Cu, and ^{67}Cu.

Such agents may be attached to the polypeptide by any suitable conventional procedure. The polypeptide comprises functional groups on amino acid side chains that can be reacted with functional groups on a desired agent to form covalent bonds, for example. Alternatively, the protein or agent may be derivatized to generate or attach a desired reactive functional group. The derivatization may involve attachment of one of the bifunctional coupling reagents available for attaching various molecules to proteins (Pierce Chemical Company, Rockford, Illinois). A number of techniques for radiolabeling proteins are known. Radionuclide metals may be attached to polypeptides by using a suitable bifunctional chelating agent, for example.

Conjugates comprising polypeptides and a suitable diagnostic or therapeutic agent (preferably covalently linked) are thus prepared. The conjugates are administered or otherwise employed in an amount appropriate for the particular application.

Polypeptides of the invention may be used in developing treatments for any disorder mediated (directly or indirectly) by defective, or insufficient amounts of the polypeptides. Further, the polypeptides of the invention may be used in developing treatments for any disorder resulting (directly or indirectly) from an excess of the polypeptide. The polypeptides of the instant invention may be administered to a mammal afflicted with such disorders.

The polypeptides may also be employed in inhibiting a biological activity of the binding partner, in *in vitro* or *in vivo* procedures. For example, a purified IL-1 eta polypeptide can be used to inhibit binding of endogenous IL-1 eta to its cell surface receptor.

Polypeptides of the invention may be administered to a mammal to treat a binding partner-mediated disorder. Such binding partner-mediated disorders include conditions caused (directly or indirectly) or exacerbated by the binding partner.

Compositions of the present invention may contain a polypeptide in any form described herein, such as native proteins, variants, derivatives, oligomers, and biologically active fragments. In particular embodiments, the composition comprises a soluble polypeptide or an oligomer comprising soluble polypeptides of the invention.

Compositions comprising an effective amount of a polypeptide of the present invention, in combination with other components such as a physiologically acceptable diluent, carrier, or excipient, are provided herein. The polypeptides can be formulated according to known methods used to prepare pharmaceutically useful compositions. They can be combined in admixture, either as the sole active material or with other known active materials suitable for a given indication, with pharmaceutically acceptable diluents (e.g., saline, Tris-HCl, acetate, and phosphate buffered solutions), preservatives (e.g., thimerosal, benzyl alcohol, parabens), emulsifiers, solubilizers, adjuvants and/or carriers. Suitable formulations for pharmaceutical compositions include those described in *Remington's Pharmaceutical Sciences*, 16th ed. 1980, Mack Publishing Company, Easton, PA.

In addition, such compositions can be complexed with polyethylene glycol (PEG), metal ions, or incorporated into polymeric compounds such as polyacetic acid, polyglycolic acid, hydrogels, dextran, etc., or incorporated into liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts or spheroblasts. Such compositions will influence the physical state, solubility, stability, rate of *in vivo* release, and rate of *in vivo* clearance, and are thus chosen according to the intended application.

The compositions of the invention can be administered in any suitable manner, e.g., topically, parenterally, or by inhalation. The term "parenteral" includes injection, e.g., by subcutaneous, intravenous, or intramuscular routes, also including localized administration, e.g., at a site of disease or injury. Sustained release from implants is also contemplated. One skilled in the pertinent art will recognize that suitable dosages will vary, depending upon such factors as the nature of the disorder to be treated; the patient's body weight, age, and general condition, and the route of administration. Preliminary doses can be determined according

to animal tests, and the scaling of dosages for human administration is performed according to art-accepted practices.

Compositions comprising polynucleotides in physiologically acceptable formulations are also contemplated. DNA may be formulated for injection, for example.

Another use of the polypeptide of the present invention is as a research tool for studying the biological effects that result from the interactions of IL-1 eta with its binding partner, or from inhibiting these interactions, on different cell types. Polypeptides also may be employed in *in vitro* assays for detecting IL-1 eta, the binding partner or the interaction thereof.

Another embodiment of the invention relates to uses of the polypeptides of the invention to study cell signal transduction. IL-1 family ligands play a central role in protection against infection and immune inflammatory responses which includes cellular signal transduction, activating vascular endothelial cells and lymphocytes, induction of inflammatory cytokines, acute phase proteins, hematopoiesis, fever, bone resorption, prostaglandins, metalloproteinases, and adhesion molecules. With the continued increase in the number of known IL-1 family members, a suitable classification scheme is one based on comparing polypeptide structure as well as function (activation and regulatory properties). Thus, IL-1 eta, like other IL-1 family ligands (IL-1α, IL-1β, and IL-18) would likely be involved in many of the functions noted above as well as promote inflammatory responses and therefore perhaps be involved in the causation and maintenance of inflammatory and/or autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease, and psoriasis. As such, alterations in the expression and/or activation of the polypeptides of the invention can have profound effects on a plethora of cellular processes, including, but not limited to, activation or inhibition of cell specific responses and proliferation. Expression of cloned IL-1 eta, or of functionally inactive mutants thereof can be used to identify the role a particular protein plays in mediating specific signaling events.

IL-1 mediated cellular signaling often involves a molecular activation cascade, during which a receptor propagates a ligand-receptor mediated signal by specifically activating intracellular kinases which phosphorylate target substrates. These substrates can themselves be kinases which become activated following phosphorylation. Alternatively, they can be adaptor molecules that facilitate down stream signaling through protein-protein interaction following phosphorylation. Regardless of the nature of the substrate molecule(s), expressed functionally active versions of IL-1 eta and its binding partners can be used to identify what substrate(s) were recognized and activated by the polypeptides of the invention. As such, these novel polypeptides can be used as reagents to identify novel molecules involved in signal transduction pathways.

Antibodies

Antibodies that are immunoreactive with the polypeptides of the invention are provided herein. Such antibodies specifically bind to the polypeptides *via* the antigen-binding sites of the antibody (as opposed to non-specific binding). Thus, the polypeptides, fragments, variants, fusion proteins, etc., as set forth above may be employed as "immunogens" in producing antibodies immunoreactive therewith. More specifically, the polypeptides, fragment, variants, fusion proteins, etc. contain antigenic determinants or epitopes that elicit the formation of antibodies.

These antigenic determinants or epitopes can be either linear or conformational (discontinuous). Linear epitopes are composed of a single section of amino acids of the polypeptide, while conformational or discontinuous epitopes are composed of amino acids sections from different regions of the polypeptide chain that are brought into close proximity upon protein folding (C. A. Janeway, Jr. and P. Travers, *Immuno Biology* 3:9 (Garland Publishing Inc., 2nd ed. 1996)). Because folded proteins have complex surfaces, the number of epitopes available is quite numerous; however, due to the conformation of the protein and steric hinderances, the number of antibodies that actually bind to the epitopes is less than the number of available epitopes (C. A. Janeway, Jr. and P. Travers, *Immuno Biology* 2:14 (Garland Publishing Inc., 2nd ed. 1996)). Epitopes may be identified by any of the methods known in the art.

Thus, one aspect of the present invention relates to the antigenic epitopes of the polypeptides of the invention. Such epitopes are useful for raising antibodies, in particular monoclonal antibodies, as described in more detail below. Additionally, epitopes from the polypeptides of the invention can be used as research reagents, in assays, and to purify specific binding antibodies from substances such as polyclonal sera or supernatants from cultured hybridomas. Such epitopes or variants thereof can be produced using techniques well known in the art such as solid-phase synthesis, chemical or enzymatic cleavage of a polypeptide, or using recombinant DNA technology.

As to the antibodies that can be elicited by the epitopes of the polypeptides of the invention, whether the epitopes have been isolated or remain part of the polypeptides, both polyclonal and monoclonal antibodies may be prepared by conventional techniques. See, for example, *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Kennet et al. (eds.), Plenum Press, New York (1980); and *Antibodies: A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1988).

Hybridoma cell lines that produce monoclonal antibodies specific for the polypeptides of the invention are also contemplated herein. Such hybridomas may be produced and identified by conventional techniques. One method for producing such a hybridoma cell line comprises immunizing an animal with a polypeptide; harvesting spleen

cells from the immunized animal; fusing said spleen cells to a myeloma cell line, thereby generating hybridoma cells; and identifying a hybridoma cell line that produces a monoclonal antibody that binds the polypeptide. The monoclonal antibodies may be recovered by conventional techniques.

The monoclonal antibodies of the present invention include chimeric antibodies, e.g., humanized versions of murine monoclonal antibodies. Such humanized antibodies may be prepared by known techniques and offer the advantage of reduced immunogenicity when the antibodies are administered to humans. In one embodiment, a humanized monoclonal antibody comprises the variable region of a murine antibody (or just the antigen binding site thereof) and a constant region derived from a human antibody. Alternatively, a humanized antibody fragment may comprise the antigen binding site of a murine monoclonal antibody and a variable region fragment (lacking the antigen-binding site) derived from a human antibody. Procedures for the production of chimeric and further engineered monoclonal antibodies include those described in Riechmann et al. (*Nature 332*:323, 1988), Liu et al. (*PNAS 84*:3439, 1987), Larrick et al. (*Bio/Technology 7*:934, 1989), and Winter and Harris (*TIPS 14*:139, May, 1993). Procedures to generate antibodies transgenically can be found in GB 2,272,440, US Patent Nos. 5,569,825 and 5,545,806 and related patents claiming priority therefrom, all of which are incorporated by reference herein.

Antigen-binding fragments of the antibodies, which may be produced by conventional techniques, are also encompassed by the present invention. Examples of such fragments include, but are not limited to, Fab and $F(ab')_2$ fragments. Antibody fragments and derivatives produced by genetic engineering techniques are also provided.

In one embodiment, the antibodies are specific for the polypeptides of the present invention and do not cross-react with other proteins. Screening procedures by which such antibodies may be identified are well known, and may involve immunoaffinity chromatography, for example.

The antibodies of the invention can be used in assays to detect the presence of the polypeptides or fragments of the invention, either *in vitro* or *in vivo*. The antibodies also may be employed in purifying polypeptides or fragments of the invention by immunoaffinity chromatography.

Those antibodies that additionally can block binding of the polypeptides of the invention to the binding partner may be used to inhibit a biological activity that results from such binding. Such blocking antibodies may be identified using any suitable assay procedure, such as by testing antibodies for the ability to inhibit binding of IL-1 eta to certain cells expressing the IL-1 eta receptors. Alternatively, blocking antibodies may be identified in assays for the ability to inhibit a biological effect that results from polypeptides of the invention binding to their binding partners to target cells. Antibodies may be assayed for the ability to inhibit IL-1 eta-mediated, or binding partner-mediated cell lysis, for example.

Such an antibody may be employed in an *in vitro* procedure, or administered *in vivo* to inhibit a biological activity mediated by the entity that generated the antibody. Disorders caused or exacerbated (directly or indirectly) by the interaction of the polypeptides of the invention with the binding partner thus may be treated. A therapeutic method involves *in vivo* administration of a blocking antibody to a mammal in an amount effective in inhibiting a binding partner-mediated biological activity. Monoclonal antibodies are generally preferred for use in such therapeutic methods. In one embodiment, an antigen-binding antibody fragment is employed.

Antibodies may be screened for agonistic (*i.e.*, ligand-mimicking) properties. Such antibodies, upon binding to cell surface receptor, induce biological effects (e.g., transduction of biological signals) similar to the biological effects induced when IL-1 binds to cell surface IL-1 receptors. Agonistic antibodies may be used to activate vascular endothelial cells and lymphocytes, induce local tissue destruction and fever (Janeway et al., 1996), stimulate macrophages and vascular endothelial cells to produce IL-6, and upregulate molecules on the surface of vascular endothelial cells.

Compositions comprising an antibody that is directed against polypeptides of the invention, and a physiologically acceptable diluent, excipient, or carrier, are provided herein. Suitable components of such compositions are as described above for compositions containing polypeptides of the invention.

Also provided herein are conjugates comprising a detectable (e.g., diagnostic) or therapeutic agent, attached to the antibody. Examples of such agents are presented above. The conjugates find use in *in vitro* or *in vivo* procedures.

The following examples are offered by way of illustration, and not by way of limitation. Those skilled in the art will recognize that variations of the invention embodied in the examples can be made, especially in light of the teachings of the various references cited herein, the disclosures of which are incorporated by reference in their entirety.

EXAMPLE 1: Isolation of the IL-1 ETA Polynucleotides

Human genomic DNA containing the an upstream portion of a cDNA disclosed in EP 0879889A2 was cloned and extended in the 3' direction. The genomic DNA was sequenced and examined for potential homology to the C-terminal section of IL-1 family members. A region with the potential to code with homology to the C-terminal section of IL-1 family members was located and is disclosed as polynucleotides 375 to 585 of SEQ. ID. NO.:1. PCR primers were synthesized containing the stop codon in the 3' or reverse primer, and the initiating ATG of the IL-1 eta cDNA (SEQ. ID. NO.:1 of EP 0879889A2) in the 5' or sense primer. Using these primers, IL-1 eta cDNA was amplified from first stand cDNA made from human tonsil mRNA. PCR was preformed using standard protocols.

EXAMPLE 2: Use of Purified IL-1 eta Polypeptides

Serial dilutions of IL-1 eta-containing samples (in 50 mM $NaHCO_3$, brought to pH 9 with NaOH) are coated onto Linbro/Titertek 96 well flat bottom E.I.A. microtitration plates (ICN Biomedicals Inc., Aurora, OH) at 100:l/well. After incubation at 4°C for 16 hours, the wells are washed six times with 200:l PBS containing 0.05% Tween-20 (PBS-Tween). The wells are then incubated with FLAG®-binding partner at 1 mg/ml in PBS-Tween with 5% fetal calf serum (FCS) for 90 minutes (100:l per well), followed by washing as above. Next, each well is incubated with the anti-FLAG® (monoclonal antibody M2 at 1 mg/ml in PBS-Tween containing 5% FCS for 90 minutes (100:l per well), followed by washing as above. Subsequently, wells are incubated with a polyclonal goat anti-mIgG1-specific horseradish peroxidase-conjugated antibody (a 1:5000 dilution of the commercial stock in PBS-Tween containing 5% FCS) for 90 minutes (100 :l per well). The HRP-conjugated antibody is obtained from Southern Biotechnology Associates, Inc., Birmingham, Alabama. Wells then are washed six times, as above.

For development of the ELISA, a substrate mix [100:l per well of a 1:1 premix of the TMB Peroxidase Substrate and Peroxidase Solution B (Kirkegaard Perry Laboratories, Gaithersburg, Maryland)] is added to the wells. After sufficient color reaction, the enzymatic reaction is terminated by addition of 2 N H_2SO_4 (50:l per well). Color intensity (indicating ligand receptor binding) is determined by measuring extinction at 450 nm on a V Max plate reader (Molecular Devices, Sunnyvale, CA).

EXAMPLE 3: Amino Acid Sequence

The amino acid sequence of IL-1 eta was determined by translation of the complete nucleotide sequences of SEQ ID NO:1.

EXAMPLE 4: DNA and Amino Acid Sequences

The nucleotide sequence of the isolated IL-1 eta and the amino acid sequence encoded thereby, are presented in SEQ ID NOs:1 and 2. The sequence of the IL-1 eta DNA fragment isolated by PCR corresponds to nucleotides 1 to 585 of SEQ ID NO:1, which encode amino acids 1 to 157 of SEQ ID NO:2.

The amino acid sequence of SEQ ID NO:2 bears significant homology to other known IL-1 ligand family members.

EXAMPLE 5: Monoclonal Antibodies That Bind Polypeptides of the Invention

This example illustrates a method for preparing monoclonal antibodies that bind IL-1 eta. Suitable immunogens that may be employed in generating such antibodies include, but are not limited to, purified IL-1 eta polypeptide or an immunogenic fragment thereof such as

the extracellular domain, or fusion proteins containing IL-1 eta (e.g., a soluble IL-1 eta/Fc fusion protein).

Purified IL-1 eta can be used to generate monoclonal antibodies immunoreactive therewith, using conventional techniques such as those described in U.S. Patent 4,411,993. Briefly, mice are immunized with IL-1 eta immunogen emulsified in complete Freund's adjuvant, and injected in amounts ranging from 10-100 g subcutaneously or intraperitoneally. Ten to twelve days later, the immunized animals are boosted with additional IL-1 eta emulsified in incomplete Freund's adjuvant. Mice are periodically boosted thereafter on a weekly to bi-weekly immunization schedule. Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision to test for IL-1 eta antibodies by dot blot assay, ELISA (Enzyme-Linked Immunosorbent Assay) or inhibition of IL-1 eta receptor binding.

Following detection of an appropriate antibody titer, positive animals are provided one last intravenous injection of IL-1 eta in saline. Three to four days later, the animals are sacrificed, spleen cells harvested, and spleen cells are fused to a murine myeloma cell line, e.g., NS1 or preferably P3x63Ag8.653 (ATCC CRL 1580). Fusions generate hybridoma cells, which are plated in multiple microtiter plates in a HAT (hypoxanthine, aminopterin and thymidine) selective medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

The hybridoma cells are screened by ELISA for reactivity against purified IL-1 eta by adaptations of the techniques disclosed in Engvall et al., (*Immunochem.* 8:871, 1971) and in U.S. Patent 4,703,004. A preferred screening technique is the antibody capture technique described in Beckmann et al., (*J. Immunol.* 144:4212, 1990). Positive hybridoma cells can be injected intraperitoneally into syngeneic BALB/c mice to produce ascites containing high concentrations of anti-IL-1 eta monoclonal antibodies. Alternatively, hybridoma cells can be grown *in vitro* in flasks or roller bottles by various techniques. Monoclonal antibodies produced in mouse ascites can be purified by ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to Protein A or Protein G can also be used, as can affinity chromatography based upon binding to IL-1 eta.

EXAMPLE 6: Northern Blot Analysis

The tissue distribution of IL-1 eta is investigated by Northern blot analysis, as follows. An aliquot of a radiolabeled riboprobe is added to two different human multiple tissue Northern blots (Clontech, Palo Alto, CA; Biochain, Palo Alto, CA). The blots are hybridized in 10X Denhardts, 50mM Tris pH 7.5, 900mM NaCl, 0.1% Na pyrophosphate, 1% SDS, 200µg/mL salmon sperm DNA. Hybridization is conducted overnight at 63°C in 50% formamide as previously described (March et al., *Nature* 315:641-647, 1985). The blots are then washed with 2X SSC, 0.1% SDS at 68°C for 30 minutes. The cells and tissues with the highest levels of IL-1 eta mRNA are determined by comparison to control probing with a β-actin-specific probe.

EXAMPLE 7: Binding Assay

Full length IL-1 eta can be expressed and tested for the ability to bind IL-1 eta receptors. The binding assay can be conducted as follows.

A fusion protein comprising a leucine zipper peptide fused to the N-terminus of a soluble IL-1 eta polypeptide (LZ-IL-1 eta) is employed in the assay. An expression construct is prepared, essentially as described for preparation of the FLAG®(IL-1 eta) expression construct in Wiley et al. (*Immunity*, 3:673-682, 1995; hereby incorporated by reference), except that DNA encoding the FLAG® peptide was replaced with a sequence encoding a modified leucine zipper that allows for trimerization. The construct, in expression vector pDC409, encodes a leader sequence derived from human cytomegalovirus, followed by the leucine zipper moiety fused to the N-terminus of a soluble IL-1 eta polypeptide. The LZ-IL-1 eta is expressed in CHO cells, and purified from the culture supernatant.

The expression vector designated pDC409 is a mammalian expression vector derived from the pDC406 vector described in McMahan et al. (*EMBO J.* 10:2821-2832, 1991; hereby incorporated by reference). Features added to pDC409 (compared to pDC406) include additional unique restriction sites in the multiple cloning site (mcs); three stop codons (one in each reading frame) positioned downstream of the mcs; and a T7 polymerase promoter, downstream of the mcs, that facilitates sequencing of DNA inserted into the mcs.

For expression of full length human IL-1 eta protein, the entire coding region (*i.e.*, the DNA sequence presented in SEQ ID NO:1) is amplified by polymerase chain reaction (PCR). The template employed in the PCR is the cDNA clone isolated from tonsil first strand cDNA, as described in example 1. The isolated and amplified DNA is inserted into the expression vector pDC409, to yield a construct designated pDC409-IL-1 eta.

IL-1 eta polypeptide is employed to test the ability to bind to host cells expressing recombinant or endogenous IL-1 eta receptors, as discussed above. Cells expressing IL-1 eta receptor are cultured in DMEM supplemented with 10% fetal bovine serum, penicillin, streptomycin, and glutamine. Cells are incubated with LZ-IL-1 eta (5 mg/ml) for about 1

hour. Following incubation, the cells are washed to remove unbound LZ-IL-1 eta and incubated with a biotinylated anti-LZ monoclonal antibody (5 mg/ml), and phycoerythrin-conjugated streptavidin (1:400), before analysis by fluorescence-activated cell scanning (FACS). The cytometric analysis was conducted on a FACscan (Beckton Dickinson, San Jose, CA).

The cells expressing IL-1 eta receptors showed significantly enhanced binding of LZ-IL-1 eta, compared to the control cells not expressing IL-1 eta receptors.

Example 8: Expression Analysis

First strand cDNAs present in Clontech (Palo Alto, CA) Human Multiple Tissue cDNA Panels I (Cat. # K1420-1) and II (Cat. #K1421-1) and the Human Immune Panel (Cat. #K1426-1) were screened by PCR amplification using sense and antisense primers. The primers were designed to span introns so that products arising from genomic DNA and cDNA could be distinguished. In some cases, nested primers were used in a second PCR reaction. The presence of an amplification product for each gene/tissue combination was determined by analysis on agarose gels stained with ethidium bromide.

Alternatively, individual cell types from human peripheral blood were isolated and stimulations were performed (Kubin et al., *Blood* 83(7):1847-55 (1994); Kubin et al., *J Exp Med* 180(1):211-22 (1994)). NK cells were incubated with IL-12 (R&D Biosystems; 1 ng/ml) for either 2 hours or 4 hours. T cells were unstimulated or stimulated with anti-CD3 (OKT-3 antibody, immobilized on plastic at 5 ng/ml) or with the combination of anti-CD3 and anti-CD28 (the anti-CD28 antibody was CD248 used in soluble form as a 1:500 dilution of ascites fluid), for 30 minutes or 4 hours. Monocytes were unstimulated, or stimulated with LPS (Sigma; 1ug/ml) for 2 or 3 hours. B cells were unstimulated, or stimulated with the combination of 0.05% SAC and 500 ng/ml CD40L trimer (Immunex) and 5 ng/ml IL-4 (Immunex) for 3.5 or 4 hours. Dendritic cells were stimulated with LPS as for monocytes, for 2 or 4 hours. After isolation of RNA and synthesis of first strand cDNA, PCR amplifications and gel analysis were performed.

Table I summarizes IL-1 eta expression data derived by PCR analysis of a panel of first strand cDNAs from Clontech. In the table, an "-" indicates that the mRNA was looked for but not found. Positive expression results are designated by an "A".

Table I

Human Tissue Source	IL-1 eta expression
Spleen	-
Lymph node	-
Thymus	-
Tonsil	A
Bone marrow	A
Fetal liver	-
Leukocyte	-
Heart	A
Brain	-
Placenta	A
Lung	A
Liver	-
Skeletal muscle	-
Kidney	-
Pancreas	-
Prostate	-
Testis	A
Ovary	-
Small intestine	-
Colon	A

What is claimed is:

1. A DNA selected from the group consisting of:
   (a) DNA comprising nucleotides 112 through 585 of SEQ ID NO:1: and
   (b) DNA encoding the amino acid sequence of SEQ ID NO:2;
   (c) DNA encoding an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO:2;
   (d) DNA that encodes a fragment of the polypeptide of SEQ ID NO:2;
   (e) DNA that is the complement of DNA that hybridizes under moderately stringent conditions to the DNA of (a) through (d);and,
   (f) DNA that is degenerate to the DNA of (a) through (e).

2. DNA comprising nucleotides 112 through 585 of SEQ ID NO:1.

3. DNA that encodes the polypeptides of SEQ ID NO:2.

4. A polypeptide selected from the group consisting of:
   a) a polypeptide comprising the amino acids sequence of SEQ ID NO:2;
   b) a polypeptide comprising an amino acid sequence that is at least 80% identical to the amino acid sequence presented in SEQ ID NO:2; and
   c) a fragment of the polypeptide of (a) or (b), wherein the fragment is capable of binding IL-1 an IL-1 receptor family member.

5. The polypeptide of SEQ ID NO:2.

6. A polypeptide encoded by the DNA of any of claims 1-3.

7. An expression vector comprising the DNA of any of claim 1 through 3.

8. A host cell transformed with an expression vector of claim 7.

9. A method for producing a polypeptide, the method comprising culturing the host cell of claim 8 under conditions that cause expression of the polypeptide.

10. An antibody immunoreactive with the polypeptide of any of claims 4 through 6.

SEQUENCE LISTING

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<110> IMMUNEX CORPORATION
      SIMS, John E.
      RENSHAW, Blair R.

<120> IL-1 ETA DNA AND POLYPEPTIDES

<130> 2932-WO

<140> --to be assigned--
<141> 2000-05-25

<150> 60/162,331
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Ser Arg Ser Ile Lys Pro Val Thr Leu His Leu Ile Ala Cys Arg Asp
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aaa gca cag aag ccc ttt ctc ttt ttc cac aat aaa gaa ggc tcc act    453
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tct gtc ttt cag tca gtc tct tac cct ggc tgg ttc ata gcc acc tcc    501
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acc aca tca gga cag ccc atc ttt ctc acc aag gag aga ggc ata act    549
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Pro Leu Ser Arg Ser Ile Lys Pro Val Thr Leu His Leu Ile Ala Cys
        35                  40                  45

Arg Asp Thr Glu Phe Ser Asp Lys Glu Lys Gly Asn Met Val Tyr Leu
    50                  55                  60

Gly Ile Lys Gly Lys Asp Leu Cys Leu Phe Cys Ala Glu Ile Gln Gly
65                  70                  75                  80

Lys Pro Thr Leu Gln Leu Lys Glu Lys Asn Ile Met Asp Leu Tyr Val
                85                  90                  95

Glu Lys Lys Ala Gln Lys Pro Phe Leu Phe Phe His Asn Lys Glu Gly
            100                 105                 110

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        115                 120                 125

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Ile Thr Asn Asn Thr Asn Phe Tyr Leu Asp Ser Val Glu
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/14435

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/25 C07K14/545 C07K16/24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

STRAND, BIOSIS, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	EP 0 879 889 A (SMITHKLINE BEECHAM CORP) 25 November 1998 (1998-11-25) cited in the application whole document especially sequences ID no.1 and no.2 —— -/--	1 2-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 August 2000	11/09/2000
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Le Cornec, N

1

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/14435

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	SMITH D.E. ET AL: EMBL DATABASE ENTRY AF201833, ACCESSION NUMBER AF201833, 16 January 2000 (2000-01-16), XP002145347 abstract -& D.E. SMITH ET AL: "Four new members expand the iL-1 superfamily" JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 275, no. 2, January 2000 (2000-01), pages 1169-1175, XP002135001 AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD., US ISSN: 0021-9258 whole document especially page 1172; figure 1; table 1	1-10

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INTERNATIONAL SEARCH REPORT

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PCT/US 00/14435

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		CA 2221866 A	19-11-1998
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		JP 2000083688 A	28-03-2000